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Three new species of Australian miracine parasitoid wasps collected by regional schools as part of the Insect Investigators citizen science project (Hymenoptera, Braconidae, Miracinae)

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Abstract

Miracinae is a poorly known and rarely collected subfamily of parasitoid wasps belonging to the family Braconidae. Here, three new species are described from material collected by Australian regional schools as part of the *Insect Investigators* citizen science project and named in collaboration with students: *Mirax supremus* Slater-Baker, **sp. nov.**, *Mirax ceduna* Slater-Baker, **sp. nov.** and *Mirax kaatijan* Slater-Baker, **sp. nov.** The barcoding region of the cytochrome c oxidase subunit I (*COI*) gene was obtained for each new species and analysed alongside all publicly available *COI* DNA barcodes for Miracinae on the Barcode of Life Database, to produce a molecular framework to guide species delimitation and efficient morphological examination. Full morphological descriptions are provided along with high quality images for each new species. A key to described Australian Miracinae is provided.

Keywords

COI, DNA barcoding, Taxonomy

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Introduction

Miracinae is a subfamily of braconid wasps which are endoparasitoids of leaf-mining lepidopteran larvae (Shaw and Huddleston 1991; Whitfield and Wagner 1991; Whitfield 1997). Miracines are characterised by the presence of a distinct Y-shaped structure formed by the arrangement of the first three metasomal tergites, antennae having 14 segments, and distinctive reduced fore wing venation (Shaw and Huddleston 1991; Whitfield 1997). The phylogenetic placement of Miracinae amongst related microgastroid subfamilies is uncertain (Murphy et al. 2008; Sharanowski et al. 2011; Jasso-Martínez et al. 2022), though they are placed sister to Cardiochilinae in the most recent phylogenetic analysis based on Ultra Conserved Elements (UCEs; Jasso-Martínez et al. 2022). Miracinae currently contains 82 species worldwide (Yu et al. 2016; Ghramh et al. 2019; Slater-Baker et al. 2022; Ranjith et al. 2023; Liu and Polaszek 2024a, b, c), and three described genera: *Mirax* Haliday 1833 (having the propodeum smooth medially, and notauli absent or reduced), *Centistidea* Rowher 1914 (having a propodeal medial longitudinal carina, and with longer, crenulated notauli) and the recently described *Rugosimirax* Ranjith & van Achterberg 2023 (with longer, crenulated notauli, and a U-shaped propodeal areola). Liu and Polaszek (2024) provide a morphological key to genera of Miracinae. The recognition of *Centistidea* as a distinct genus has been variable (Muesebeck 1922; Whitfield 1997; Papp 2013; Ghramh et al. 2019; Ranjith et al. 2023), and recent descriptions of Miracinae are continually revealing morphological diversity and plasticity within the subfamily (Ranjith et al. 2023; Liu and Polaszek 2024). Here, we treat *Centistidea* as a synonym of *Mirax* following previous work on Australian Miracinae (Slater-Baker et al. 2022).

In the first formal documentation of Miracinae in Australia (Slater-Baker et al. 2022), three species were described from material collected in South Australia, whilst a fourth species was described from Queensland in northern Australia. Many putative species could not be formally described by Slater-Baker et al. (2022), due to a lack of female specimens of sufficient quality for morphological examination. The three new species presented here are described from female miracine wasps recently collected in collaboration with Australian remote-area schools and DNA barcoded as part of the *Insect Investigators* citizen science project (https://insectinvestigators.com.au/). This project involved collaboration with 50 regional schools across the states of South Australia, New South Wales and Queensland, which conducted insect surveys using Malaise traps for four weeks. The wide-reaching efforts of *Insect Investigators* resulted in collection of thousands of insect specimens from under-sampled regions of Australia (Barcode of Life project code ASMII; https://www.boldsystems.org/).

Here, full morphological descriptions are provided for three new species of Australian Miracinae, along with cytochrome c oxidase subunit I (*COI*) DNA barcodes and high-resolution, focus-stacked images to aid diagnosis. As is now standard for integrated species descriptions of Hymenoptera (Butcher et al. 2012; Martinez et al. 2012; Kocić et al. 2020; Olmi et al. 2022; Liu et al. 2023; Sharkey et al. 2023; Jasso-Martínez et al. 2024), *COI* DNA barcodes are used to support species descriptions using molecular species delimitation. A *COI* tree is produced as a visual aid to assess relatedness between DNA barcodes. New species presented here are named

in collaboration with the schools involved in collecting the type material, with the goal of introducing students to the topics of taxonomy and insect diversity, and inspiring emerging scientists as part of the *Insect Investigators* citizen science project (https://insectinvestigators.com.au/).

Methods

Collection and DNA barcoding

Holotypes for new species described here were collected as part of the *Insect Investigators* citizen science project via Malaise trapping. Specimens were sent to the Canadian Centre for DNA Barcoding (CCDB) at The University of Guelph, Canada, to undergo whole-body non-destructive DNA extraction and sequencing of the barcoding region of the *COI* gene (Hebert et al. 2003) using CCDB protocols. Specimens were returned to Australia in their entirety for morphological examination, and deposited in Australian collections.

Additional specimens examined were collected and barcoded prior to this project. The specimen represented by BOLD Sample ID AUMIC889-23 underwent DNA extraction from a hind leg using a modified version of the Canadian Centre for DNA Barcoding Glass Fibre Plate DNA Extraction Protocol (Ivanova et al. 2006), followed by DNA library preparation for amplicon-based Illumina Miseq high-throughput sequencing (Cruaud et al. 2017), and sequencing by the Australian Genome Research Facility (AGRF). Collection and sequencing methods for other Australian Miracinae examined are described by Slater-Baker et al. (2022).

Public *COI* data

All available *COI* sequences identified as Miracinae as of May 2023 were downloaded from BOLD (n = 451), excluding sequences flagged as contaminants, containing stop codons, misidentifications/errors, and sequences of < 100 bp length. Fourteen of these sequences represent specimens collected in Australia, including specimens of the three new species described here. To account for uncertainty in the phylogenetic placement of Miracinae (Murphy et al. 2008; Sharanowski et al. 2011; Jasso-Martínez et al. 2022), eight *COI* sequences identified as belonging to the related subfamilies Cardiochilinae, Cheloninae and Microgastrinae were selected and included as outgroup taxa. All BOLD codes for sequences used in the analysis are listed in Suppl. material 1.

Tree reconstruction

A *COI* tree was produced to efficiently visualise relatedness between DNA barcodes for Miracinae. Sequences were aligned using MAFFT v7.490 (Katoh et al. 2002; Katoh and Standley 2013) with default settings in Geneious Prime version 2023.0.4 (https://www.geneious.com). The alignment was manually inspected and translated within Geneious

Prime to check for the presence of indels, stop codons and misaligned regions. A maximum likelihood tree was produced for the *COI* alignment using IQ-TREE v2.3.5 (Minh et al. 2020) with 1000 ultrafast bootstrap (Minh et al. 2013) replicates, and 1000 SH-aLRT (Guindon et al. 2010) replicates. A best-fit substitution model was selected using ModelFinder (Kalyaanamoorthy et al. 2017), with separate partitions for each codon position and partition merging enabled. The analysis was repeated four times with identical settings to compare log likelihood values and BIC model fit values of alternate tree typologies. The model test with the lowest BIC score amongst the four replicates suggested the following partitioning scheme: codon 1, TIM+F+R4; codon 2, TIM+F+I+G4; codon 3, TIM3+F+R5. Trees were rooted and edited using FigTree v143. The root was placed at the branch leading to Cheloninae, as this subfamily is proposed to be sister to all other microgastroid subfamilies (Murphy et al. 2008; Jasso-Martínez et al. 2022).

Molecular species delimitation

Species are defined in this study as distinct evolutionary lineages, as per the General Lineage Species Concept (de Queiroz 1998). Molecular species delimitation was performed using a 2% *COI* difference threshold. This method is well established for species delimitation in the closely related subfamily Microgastrinae (Smith et al. 2008; Smith et al. 2013; Fagan-Jeffries et al. 2018; Fagan-Jeffries et al. 2022), and used for previous work delimiting species of Miracinae (Slater-Baker et al. 2022). The 2% *COI* difference threshold was applied manually for Australian specimens according to pairwise distances calculated for the *COI* barcode alignment using two methods (see Suppl. material 2): percent identity distance values calculated in Geneious Prime version 2023.0.4 via the 'distances' tab for the alignment; and Kimura two-parameter model (K2P; Kimura 1980) corrected distances calculated from the same alignment in MEGA 11 version 11.0.13 using the invertebrate mitochondrial genetic code option, K2P model selection, and all other settings default. The 2% *COI* distance threshold delimitation was compared with results from the Assemble Species by Automatic Partitioning (ASAP; Puillandre et al. 2021) species delimitation tool. The ASAP tool was run via the web implementation (https://bioinfo.mnhn.fr/abi/public/asap/) using both K2P and simple distances (P-distances) for the Miracinae *COI* alignment with outgroups removed. The partitions receiving the lowest ASAP score were selected for final delimitation results, after examining results and probability values for alternative highly ranked partitions. Putative species based on molecular delimitation results were examined morphologically and diagnosed against described species of Miracinae from Australia.

Morphological examination

Specimens were initially stored in 100% ethanol after DNA extraction, and chemically dried using Hexamethyldisilazane (HMDS) following Heraty and Hawks (1998), with the following modifications: initial soak in HMDS for 30 mins, second soak for 45 mins. Additional specimens examined in this study were previously dried directly

from ethanol (see Slater-Baker et al. (2022) for further details and images). Chemical drying was employed here to better maintain specimen quality by reducing the degree of collapsing, especially in the shape of the metasoma. This facilitated more efficient morphological examination and minimised specimen handling, which is preferable for miracine wasps due to their delicateness. Specimens were mounted on card points and examined using an Olympus SZX16 stereo microscope. The morphological characters chosen for description were based on commonly used characters in existing literature describing miracine wasps (Wu et al. 2000; Beyarslan 2009; Papp 2013; Cauich-Kumul et al. 2014; Farahani et al. 2014; Ghramh et al. 2019; Ranjith et al. 2019; Slater-Baker et al. 2022) to allow for comparison to previously described species. Methods used for measuring morphological characters of Miracinae follow Slater-Baker et al. (2022).

Imaging

Images of specimens were captured using a Canon EOS 5DS R with MP-E65 mm macro lens, and Stackshot macro rail and controller. Focus-stacking of images was performed in Zerene Stacker 1.0.4. Note that all specimens were imaged after whole-body DNA extraction, and remain in good condition. Previous work on Australian Miracinae utilised Scanning Electron Microscopy (SEM) to characterise diagnostic sculptural details such as the medio-posterior depressions of the scutellum. SEM was deemed unnecessary for this project, however, as all morphological features examined could be adequately observed using the Olympus SZX16 microscope, while reducing excessive handling required for SEM.

Outreach

Throughout the process of species description, we took opportunities to engage with schools to impart knowledge of the taxonomic process relevant to students' role in describing species, and to foster a sense of ownership/connection with the species wherever possible. This was done using a two-step iterative process: Firstly, 1a) in-person and/or online hybrid workshops were run with students involved in collecting the type material of each new species described here. During workshops, taxonomists supplied students with background information on miracine wasps, including parasitoid wasp biology, morphological features related to the undescribed species, and rules and instructions for scientific naming; 1b) school teachers facilitated students to brainstorm names using an electronic notice board (Padlet), including reasons behind the suggested names. Secondly, 2a) taxonomists subsequently filtered name ideas on the notice board and adjusted grammar to provide a collaborative shortlist of suitable names to the teachers and students, along with educational information about why certain names were selected or not (e.g. avoiding names based on specimen preservation or features likely to be variable); 2b) where possible, teachers facilitated students to select a consensus species epithet via a vote amongst the students and in consultation with the school community. In one instance, a final name was selected by the authors based on students' suggestions.

Terminology

General morphological terminology follows the Hymenoptera Anatomy Ontology preferred terms (Yoder et al. 2010; specific definitions are given in Suppl. material 3), with terminology for wing venation following Sharkey and Wharton (1997). Terms for sculpturing follow Eady (1968).

Abbreviations

M/T Malaise trap
OOL ocular-ocellar line
POL posterior inter-ocellar line
POD posterior ocellus diameter
T1 first mediotergite
T2 second mediotergite
QM Queensland Museum, Brisbane, Australia
SAMA South Australian Museum, Adelaide, Australia
WAM Western Australian Museum, Perth, Australia
ANIC Australian National Insect Collection, Canberra, Australia

Results

COI barcoding and molecular species delimitation

Of the four tree reconstruction analyses undertaken on the final *COI* alignment, the tree with the highest log likelihood value and lowest BIC score is shown in Fig. 1. Australian Miracinae are observed to form two main clades in Fig. 1, with the exception of the undescribed species represented by BOLD Process ID: OZBOL411-21. One of the three alternative analysis replicates shows similar tree topology to Fig. 1 for the Australian species, whilst in contrast the remaining two analyses showed Australian specimens formed a single clade with the exception of BOLD Process ID: OZBOL411-21. The *COI* DNA barcodes of Australian miracines described here were found to be distinct from *COI* barcodes for specimens obtained outside of Australia in the global BOLD dataset (Fig. 1. see full tree in Suppl. material 4). Australian specimens examined show morphological characters typically attributed to both *Centistidea* and *Mirax* (see: Liu and Polaszek (2024)), however the *COI* tree estimated here did not consistently separate lineages displaying these morphologies, with one clade containing Australian specimens displaying a strong propodeal medial longitudinal carina, and the other containing a mixture of specimens with and without a propodeal medial longitudinal carina (Fig. 1). This pattern is consistent in all alternative runs of the tree reconstruction (see Suppl. material 5). Additionally, *COI* barcodes for specimens identified on BOLD as *Centistidea mogrus* (Papp, 1987) from Japan do not appear to be closely related to Australian Miracinae which

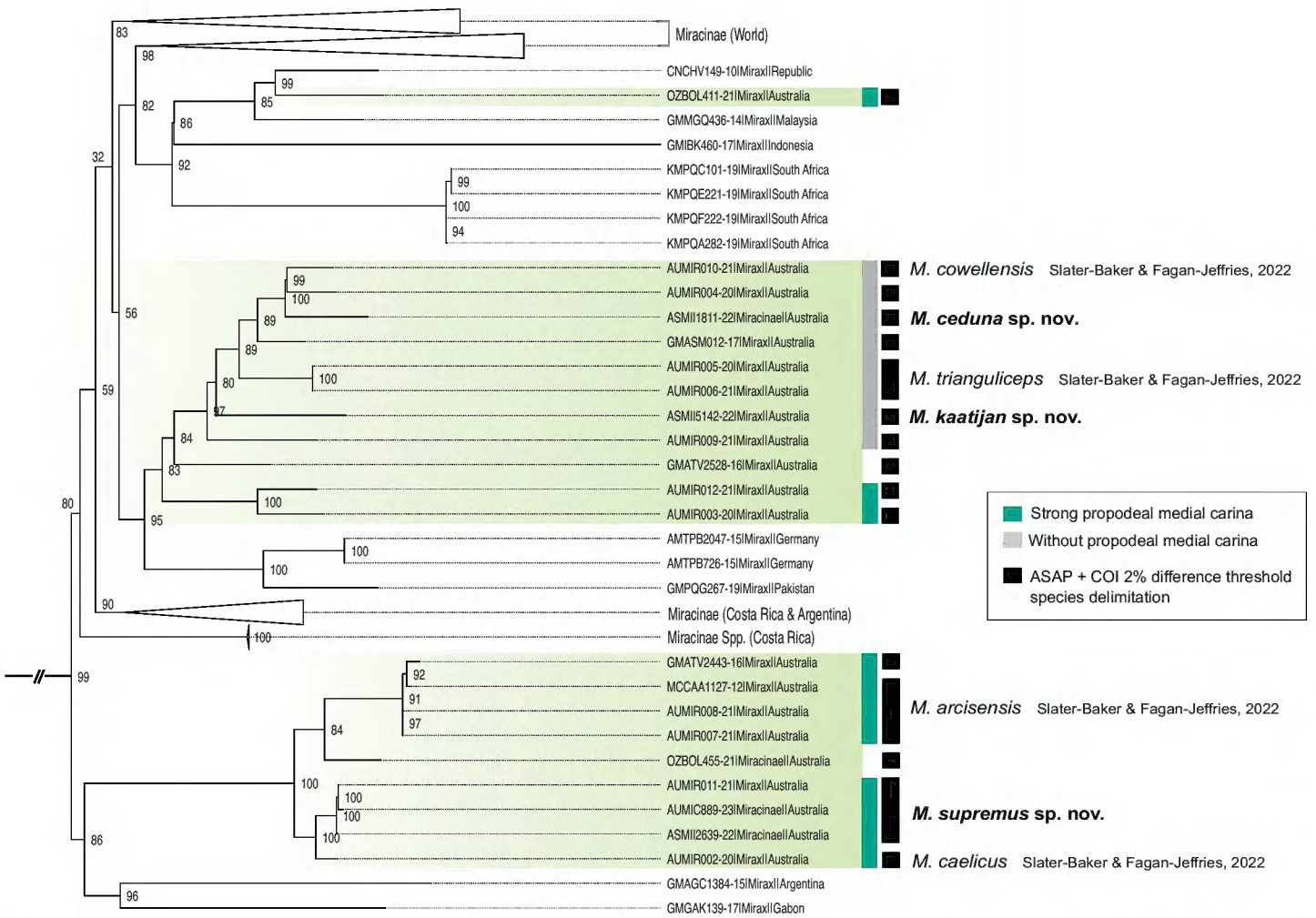

Figure 1. Maximum Likelihood *COI* tree of Miracinae, focusing on specimens collected in Australia. Ultrafast bootstrap support values are shown beside major nodes. Morphological character state of the propodeum, and a summary of species delimitation results are shown on the right. A full version of this tree is provided in Suppl. material 4.

display a distinct propodeal medial longitudinal carina typical of the genus *Centistidea* (See Suppl. material 4).

Species delimitation results suggest that the three newly collected Australian Miracinae specimens each represent a distinct species. For the Australian specimens, ASAP and 2% *COI* difference threshold methods agreed on putative species groupings in all cases (Fig. 1; see full results in Suppl. materials 2, 5), and each species grouping represents its own BOLD BIN at the time of study. Molecular species groupings for the three new species described here corresponded with differences in morphology in all cases, though due to highly conserved morphology displayed by Miracinae, and a limited ability to assess intraspecific variation in this study, some species are best differentiated based on *COI* barcode divergence. The newly collected female holotype of *Mirax supremus* sp. nov. forms a monophyletic group with two specimens previously collected from Australia, which were morphologically examined and are included in the type series. The other two new species described here were not represented amongst previously barcoded Australian miracines (Slater-Baker et al. 2022), and represent new BIN records for BOLD. Whilst these two species are represented by single specimens, we believe it is valid to describe them because the inclusion of molecular data will allow additional specimens to be associated easily in the future. Additionally, the new descriptions provide the op-

portunity to involve regional school students in formally documenting their local fauna. Australian lineages consisting of only male specimens, or specimens not available at the time of study are not treated in the present study. Full tree reconstruction and species delimitation results are available in supplementary material (See Suppl. materials 4, 5).

Taxonomy

Mirax Haliday, 1833

Mirax Haliday, 1834: 263. See key references: Haliday 1834:230; Haliday 1835:467; Ashmead 1900:131; Muesebeck 1922:10; Papp 2013:97. For full reference list up to 1973, see Shenefelt 1973:676.

Centistidea Rowher, 1914:81. See key reference: van Achterberg and Mehrnejad 2002:32. For full reference list up to 1973, see Shenefelt 1973:676.

Type species. *Mirax rufilabris* Haliday, 1833:263, description in Haliday 1834:230

Remarks. We treat *Centistidea* as a synonym of *Mirax* based on preliminary molecular tree reconstructions presented here and in Slater-Baker et al. (2022), as well as an observed continuous range of presence to absence of the propodeal medial longitudinal carina in Miracinae. No molecular data are available for specimens identified as the genus *Rugosimirax*, therefore, all specimens assigned to the genus *Mirax* in this study were morphologically diagnosed against *Rugosimirax* using the keys to the genera of Miracinae provided by Ranjith et al. (2023) and Liu and Polaszek (2024).

We acknowledge that the treatment of genera used in this study may require revision following improved understanding of the genera of Miracinae based on molecular data. We chose to take a conservative approach supported by the preliminary molecular results presented here and in previous work (Slater-Baker et al. 2022), and focus on the goal of continued species-level documentation of Miracinae in Australia.

Key to described Australian Miracinae

The following key separates female specimens of the seven currently described species of Australian Miracinae. Because Miracinae is expected to be diverse within Australia, there are likely to be many undescribed Australian species not considered in this key. Additionally, due to the rarity of collection of Miracinae, the key may not adequately account for morphological variation within species. We therefore strongly recommend the use of DNA barcoding for the identification of Australian Miracinae. Alternatively, identifications made using this key should be supported by careful comparison to type material.

1 Propodeum smooth medially, without a medial longitudinal carina (Figs 2D, 3D)..... **2**

– Propodeum with a medial longitudinal carina, meeting transverse carinae in the posterior half (Fig. 4D) **5**

2 Head and mesosoma colouration mostly black or brown (Figs 2A, 4A).....**3**
– Head and mesosoma colouration mostly yellow (Fig. 3A). [Scutellar medio posterior depressions semi-elliptical, separated by a distance approximately equal to the width of one depression (Fig. 3D): scape and pedicel yellow-brown, notably paler than the flagellum (Fig. 3A, E)] .....***M. kaatijan***
3 Dorsal head at least 2× wider than medial length; dorsal medial head length < 1.3× longer than dorsal eye length (maximum length measured diagonally; see Slater-Baker et al. 2022 for measurement methods); when viewed anteriorly, the head appears sub-triangular in shape, with the eyes bulged in the dorsal half (Fig. 2E, 5C) **4**
– Dorsal head approximately 1.7× wider than medial length; dorsal medial head length approximately 1.5× longer than dorsal eye length (maximum length measured diagonally); head when viewed anteriorly appears nearly ovoid (Fig. 5A, B) .....***M. cowellensis***
4 Scape, pedicel and first flagellomere yellow-brown (Fig. 5C); dorsal head sparsely setose, face densely setose laterally, but without setae medially (Fig. 5C); inner eye margin approximately parallel (Fig. 5C).....***M. trianguliceps***
– Scape, pedicel and first flagellomere dark brown (Fig. 2E); dorsal head and face densely setose throughout (Fig. 2E, F); inner eye margin narrowing slightly posteriorly (towards clypeus; Fig. 2E) .....***M. ceduna***
5 T1 relatively broad, < 3× longer than maximum width (Fig. 6A, B); scutelllar medio-posterior depressions comparatively larger (Fig. 6D, E). Note: these two species are morphologically very similar and are best identified based on *COI* barcodes.....***M. caelicus or M. supremus***
– T1 relatively narrow, > 3× longer than wide, and strongly narrowed basally (Fig. 6C); scutellar medio-posterior depressions comparatively smaller (Fig. 6F) .....***M. arcisensis***

***Mirax ceduna* Slater-Baker, sp. nov.**
https://zoobank.org/E9022279-58DE-40F7-884B-342AB86369EA
Fig. 2

Specimens examined. ***Holotype*:** Australia • 1 ♀; South Australia, Ceduna; 32°08.22'S, 133°0.26'E; 15–22 Mar. 2022; Ceduna Area School students leg.; M/T; BOLD Sample ID: BIOUG84494-A06; BOLD Process ID: ASMII1811-22; SAMA 32-49901

Diagnosis. This species can be differentiated from *M. cowellensis* by the dimensions of the head in dorsal view, with dorsal head width/medial length being 2.1 in *M. ceduna*, compared to 1.7 in *M. cowellensis*. When viewed anteriorly, the head appears sub-triangular in shape, with the eyes more bulged in the dorsal half in *M. ceduna*, whist the anterior head appears ovoid in *M. cowellensis*. *M. ceduna* may be separated from *M. trianguliceps* by the following characters: scape, pedicel and basal flagellomeres dark brown in *M. ceduna*, as opposed to yellow-brown in *M. trianguliceps*; dorsal head and

face densely setose throughout in *M. ceduna*, whilst dorsal head is sparsely setose, and medial region of the face lacks setae in *M. trianguliceps*; inner eye margin narrowing slightly posteriorly (towards clypeus) in *M. ceduna*, whereas it is approximately parallel in *M. trianguliceps*. These species may be best separated based on DNA barcodes for which the holotype of *M. ceduna* is 8.6% and 11.5% divergent from holotypes of *M. cowellensis* and *M. trianguliceps* respectively (Fig. 1; See Suppl. material 2). *M. ceduna* can be distinguished from all other described Australian miracines (*M. arcisensis*, *M. caelicus*, *M. kaatijan*, *M. supremus*) by the absence of a propodeal medial longitudinal carina.

Description. ***Size*:** body length 1.4 mm; fore wing length 1.4 mm; length of antenna slightly shorter than body length (Fig. 2A).

***Colour*:** head and mesosoma dark brown, except for yellow mandibles with brown tip and dull yellow mouthparts; metasoma dull yellow basally, gradating to brown distally; T1 yellow with brown margin; T2 yellow; ovipositor sheaths brown; hind coxa, femur, tibia and tarsus yellow-brown; fore wing veins and pterostigma translucent yellow.

***Head*:** dorsal head 2.1× wider than medial length (Fig. 2F); dorsal head width 1.8× face height (Fig. 2E, F); head and face smooth, densely setose throughout (Fig. 2E, F); head shape sub-triangular in anterior view (Fig. 2E); dorsal eye length (maximum length measured diagonally) 0.7× distance between the eyes at narrowest point (Fig. 2F); dorsal medial head length 1.2× longer than dorsal eye length; distance between the eyes at narrowest point 0.6× head width in dorsal view (Fig. 2F); ratio POD:POL:OOL = 1 : 2.8 : 3.6 (Fig. 2F); inner eye margin narrowing slightly posteriorly (towards clypeus; Fig. 2E); eyes with a few short, sparse setae (barely visible); antennae with 14 segments; scape 1.6× longer than wide; pedicel 1.7× longer than wide; first flagellomere 3.5× longer than wide; 11th flagellomere 2.1× longer than wide; apical flagellomere pointed; distal end of each flagellomere with several thickened setae which are longer than surrounding setae.

***Mesosoma*:** mesosoma 0.4× body length, 1.5× longer than wide; anteromesoscutum mostly smooth, densely setose; scutellar sulcus faintly indicated by smooth, shallow depression (Fig. 2D); scutellum with small–medium, semi-elliptical mediposterior depressions, separated by distance approximately equal to one depression (Fig. 2D); propodeum mostly smooth (Fig. 2D).

***Wings*:** pterostigma 2.2× longer than wide, with outer edge rounded and protruding slightly from wing outline (Fig. 2C); length of vein 2RS 4.8× r-rs, vein 2-M 2.7× longer than r-rs (Fig. 2C).

***Legs*:** hind coxa, femur and tarsus densely setose, except for basal hind coxa sparsely setose; length of hind femur 2.1× hind basitarsus; length of hind tibia 2.9× hind basitarsus.

***Metasoma*:** metasoma 0.5× body length; T1 approximately 2.1× longer than maximum width, teardrop shaped, rounded apically; T1 smooth, with a few setae distally; ovipositor sheaths short, 2.7× longer than wide, densely setose (Fig. 2G).

Male. Unknown.

Remarks. As of publication, this species forms BOLD BIN: BOLD:AES9162 and is 7.69% divergent from its nearest neighbour based on *COI* on BOLD. The holotype is deposited at the South Australian Museum, Australia.

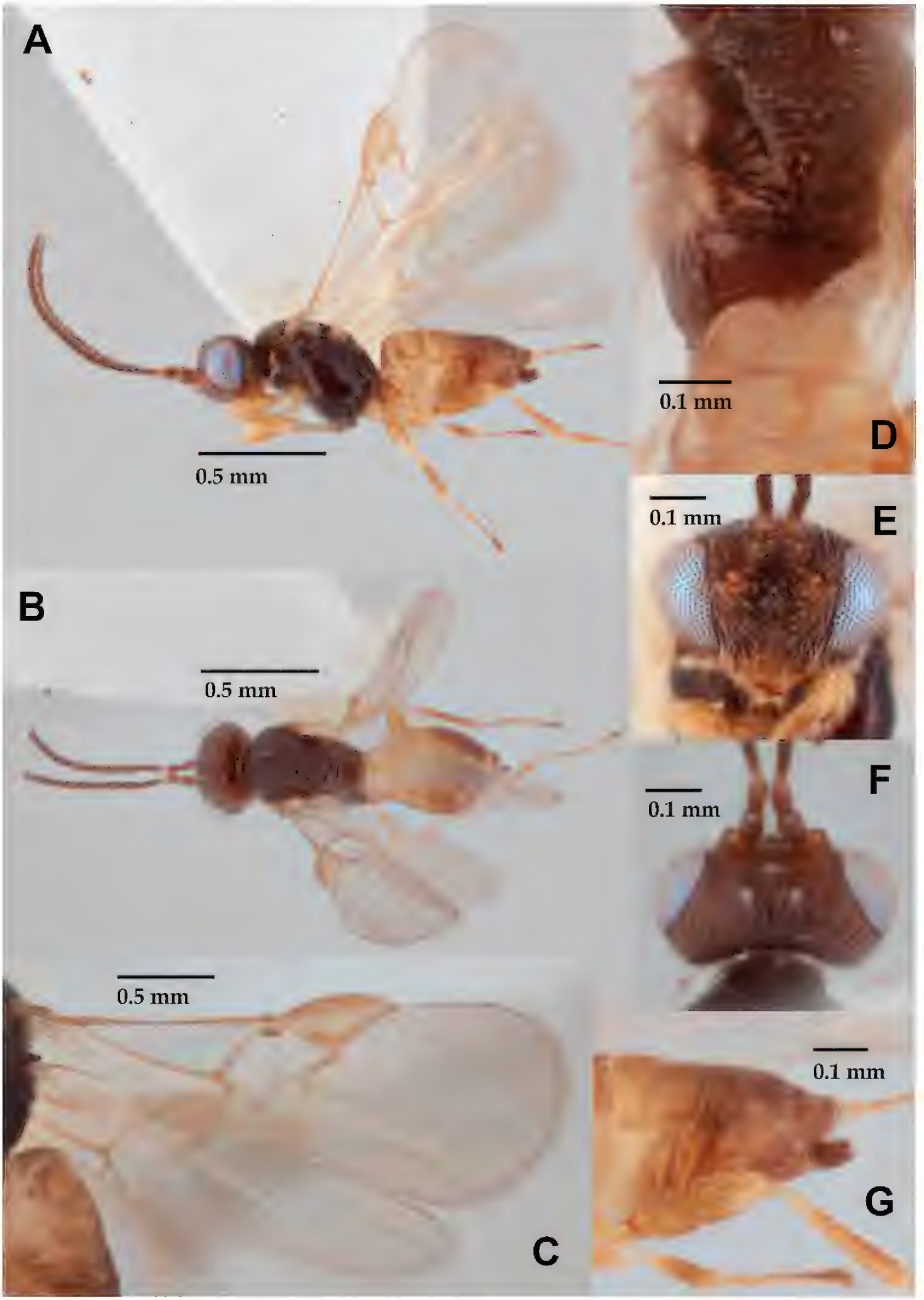

Figure 2. *Mirax ceduna* holotype **A** lateral habitus **B** dorsal habitus **C** wings **D** dorsal view of scutellar medio-posterior depressions and propodeum **E** anterior head **F** dorsal head **G** lateral posterior metasoma showing ovipositor and sheaths.

Etymology. This species was named in honour of the collection locality and Ceduna Area School students who collected the specimen. This species is colloquially known by the students as the 'golden-bum wasp', however a collaborative decision was made to have the formal scientific name be more broadly relevant to the local community. The epithet 'ceduna' is a noun in apposition.

Distribution. This species is currently known from Ceduna, South Australia, however may be found in other parts of Australia. Further sampling is required to determine an accurate distribution for this species.

Mirax kaatijan Slater-Baker, sp. nov.

https://zoobank.org/55738A4E-14F6-4F8D-BD12-61F518681DF8

Fig. 3

Specimens examined. ***Holotype*:** Australia • 1 ♀; Western Australia, William Bay National Park; 35°01.14'S, 117°13.68'E; 22–29 Mar. 2022; Kwoorabup Nature School students leg.; M/T; BOLD Sample ID: BIOUG82737-C06; BOLD Process ID: ASMII5142-22; WAM E111731

Diagnosis. *Mirax kaatijan* can be distinguished from all described Australian Miracinae by the solid yellow colouration of the head and mesosoma. *Mirax kaatijan* appears similar to an undescribed Australian species collected from Gleneagle State Forest WA, represented by BOLD process ID: AUMIR010-21, however this undescribed male specimen has the mesosoma mostly brown, and scutellar medio-posterior depressions large and ovoid, separated by a distance less than one depression.

Description. ***Size*:** body length 1.5 mm; fore wing length 1.7 mm; length of antenna approximately equal to body length (Fig. 3A).

***Colour*:** head and mesosoma yellow, except for flagellum and tip of mandible dark brown. metasoma dull yellow basally, gradating to brown distally; T1 yellow with yellow-brown margin; T2 yellow; ovipositor sheaths brown; hind coxa, femur, tibia and tarsus yellow; fore wing veins and pterostigma translucent brownish.

***Head*:** dorsal head 1.6× wider than medial length (Fig. 3F); dorsal head width 1.3× face height (Fig. 3E, F); head and face smooth, with short, dense setae throughout (Fig. 3E, F); head shape nearly ovoid in anterior view (Fig. 3E); dorsal eye length (maximum length measured diagonally) 0.7× distance between the eyes at narrowest point (Fig. 3F); dorsal medial head length 1.5× longer than dorsal eye length; distance between the eyes at narrowest point 0.6× head width in dorsal view (Fig. 3F); ratio POD:POL:OOL = 1 : 1.7 : 2.9 (Fig. 3F); inner eye margin narrowing slightly posteriorly (towards clypeus; Fig. 3E); eyes with a few short, sparse setae (barely visible); antennae with 14 segments; scape 1.9× longer than wide; pedicel 2.1× longer than wide; first flagellomere 3.9× longer than wide; 11th flagellomere 1.9× longer than wide; apical flagellomere pointed; distal end of each flagellomere with several thickened setae which are longer than surrounding setae.

***Mesosoma*:** mesosoma 0.4× body length, 1.6× longer than wide; anteromesoscutum mostly smooth, densely setose; scutellar sulcus faintly indicated by smooth, shallow depression and band of yellow-brown colouration (Fig. 3D); scutellum with

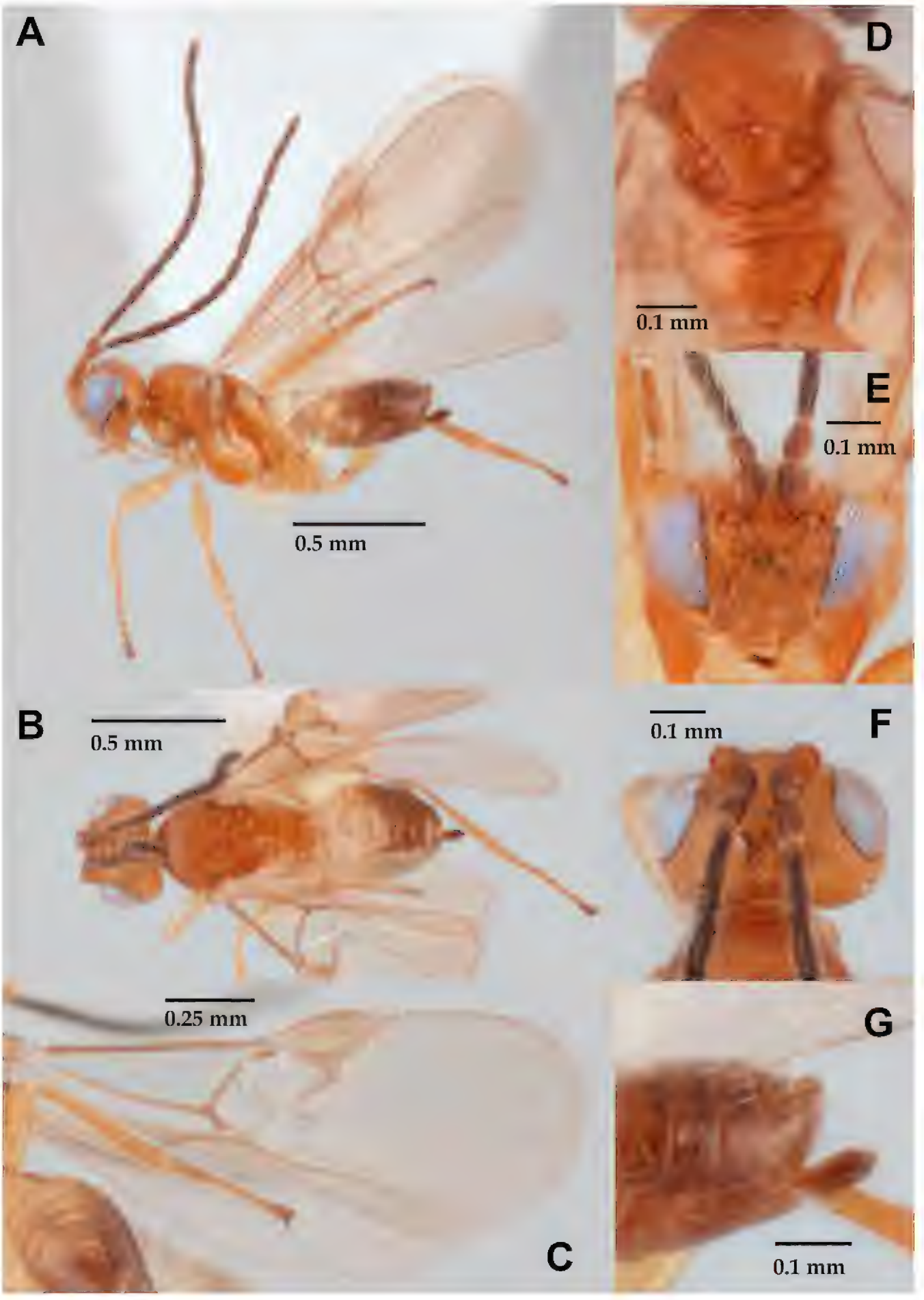

Figure 3. *Mirax kaatijan* holotype **A** lateral habitus **B** dorsal habitus **C** wings **D** dorsal view of scutellar medio-posterior depressions and propodeum **E** anterior head **F** dorsal head and mesoscutellum **G** lateral posterior metasoma showing ovipositor and sheaths.

medium-sized, semi-elliptical medio-posterior depressions, separated distance approximately equal to one depression (Fig. 3D); propodeum mostly smooth, without medial longitudinal carina (Fig. 3D).

***Wings*:** pterostigma 2.3× longer than wide, with outer edge rounded and protruding slightly from wing outline (Fig. 3C); length of vein 2RS 4.5× r-rs, vein 2-M 1.9× longer than r-rs (Fig. 3C).

***Legs*:** hind coxa, femur and tarsus densely setose, except for basal hind coxa sparsely setose; length of hind femur length 1.7× hind basitarsus; length of hind tibia 2.1× hind basitarsus.

***Metasoma*:** metasoma 0.4× body length; T1 approximately 1.9× longer than maximum width, teardrop shaped, rounded apically; T1 smooth, with a few setae distally; ovipositor sheaths short, 2.2× longer than wide, densely setose (Fig. 3G).

Male. unknown.

Remarks. As of publication, this species forms BOLD BIN: BOLD:AES5214 and is 11.22% divergent from its nearest neighbour based on *COI* on BOLD. The Holotype is deposited in the Western Australian Museum, Australia.

Etymology. The species epithet 'kaatijan' means 'knowledge' or 'learning' in the Noongar language of southwestern Western Australia. The name was selected by Kwoorbup Nature School students in collaboration with Menang Noongar Elder Uncle Lester Coyne to signify the importance of knowledge about our insects, as well as the students' learning throughout the *Insect Investigators* project. The epithet 'kaatijan' is a noun in apposition.

Distribution. This species is currently known only from William Bay National Park, WA, however may be found in other parts of Australia. Further sampling is required to determine an accurate distribution for this species.

***Mirax supremus* Slater-Baker, sp. nov.**
https://zoobank.org/C2A89CD4-93FE-4755-9DD9-EF7DCAC63842
Fig. 4

Specimens examined. ***Holotype***: Australia • 1 ♀; Queensland, Beerwah; 26°51.93'S, 152°57.22'E; 1–8 Mar. 2022; Beerwah State High School students leg.; M/T; MS22-2; BOLD Sample ID: BIOUG82726-D01; BOLD Process ID: ASMII2639-22; QM T261159. ***Paratypes*:** Australia • 1 ♀; Queensland, Kuranda, 16°48.81'S, 145°38.59'E; 317 m; 4 Sept. – 29 Oct. 2020; M.S. Moulds leg.; M/T; BOLD Sample ID 22-ME334; BOLD Process ID: AUMIC889-23; QM T261160 • 1 ♂; New South Wales, Barren Grounds; 34°40.18'S, 150°42.72'E; 23–29 Jan. 2020; K. Bayless, J. Lumbers leg.; M/T; BOLD Sample ID: ExtractionMS4; BOLD Process ID: AUMIR011-21; ANIC: 32-085575.

Diagnosis. *Mirax supremus* is morphologically very similar to its closest known relatives *M. caelicus* and *M. arcisensis*. *M. supremus* is therefore best distinguished from these species based on DNA barcodes, for which the holotype of *M. supremus* is 4.6 and 9.3% divergent from holotypes of *M. caelicus* and *M. arcisensis* respectively (Fig. 1; see Suppl. material 2). There are some subtle morphological differences between these species however: T1 is comparatively broader (2.1–2.4× longer than maximum width) in *M. supremus*, whereas it is 3.2–3.4× longer than wide and appears strongly narrowed basally in *M. arcisensis*; Scutellar medio-posterior depressions are smaller and semi-eliptical in *M. arcisensis*,

whereas they are larger and oval-shaped in *M supremus*; Scutellar medio-posterior depressions are separated by a distance approximately equal to the maximum width of one depression in *M. supremus*, whereas they separated by a distance less than the maximum width of one depression in both *M. caelicus* and *M. arcisensis*; Ratio of r-rs/2RS length is 4.4–4.3 in *M. supremus*, whereas it is 3.7 *in M. caelicus. M. supremus* can be differentiated from all other described Australian Miracinae (*M. ceduna*, *M. cowellensis*, *M. kaatijan*, *M. trianguliceps*) by the presence of a medial longitudinal carina on the propodeum.

Description. Measurements of the holotype are provided, with paratype measurements given in parentheses when different to the holotype.

***Size*:** body length 1.3 mm; fore wing length 1.4 (1.5) mm; length of antenna approximately equal to body length (Fig. 4A).

***Colour*:** head and mesosoma dark brown, except for mandible yellow-brown (or brown), mouthparts dull yellow, scape and pedicel brown; metasoma dull yellow basally, gradating to brown distally; T1 yellow-brown (or brown), with brown (or dark) margin; T2 yellow; ovipositor sheaths brown; hind coxa, femur and tarsus yellow; fore wing veins and pterostigma translucent brown.

***Head*:** dorsal head 1.9 (1.8) × wider than medial length (Fig. 4F); dorsal head width 1.7 (1.5) × face height (Fig. 4E, F); head and face smooth, sparsely setose dorsally, with the face densely setose (Fig. 4E, F); head shape nearly ovoid in anterior view (Fig. 4E); dorsal eye length (maximum length measured diagonally) 0.7× dorsal distance between the eyes at narrowest point (Fig. 4F); dorsal medial head length 1.3× longer than dorsal eye length; dorsal distance between the eyes at narrowest point 0.6× head width in dorsal view (Fig. 4F); ratio POD:POL:OOL; 1:2:3.5 (1:1.9:3.2) (Fig. 4F); inner eye margin narrowing slightly posteriorly (towards clypeus; Fig. 4E); eyes with a few short, sparse setae (barely visible); antennae with 14 segments; scape 2.0 (1.9) × longer than wide; pedicel 1.9 (1.8) × longer than wide; first flagellomere 5.0 (4.5) × longer than wide; 11th flagellomere 2.2 (2.6) × longer than wide; apical flagellomere pointed; distal end of each flagellomere with several thickened setae which are longer than surrounding setae.

***Mesosoma*:** mesosoma 0.4 (0.3) × body length, 1.4 (1.2) × longer than wide; anteromesoscutum mostly smooth (or with very shallow, dense punctures, more prominent towards edges), moderately–densely setose; scutellar sulcus faintly indicated by smooth, shallow depression (Fig. 4D); scutellum with medium-sized, elongate, oval-shaped medio-posterior depressions, separated by width approximately equal to one depression (Fig. 4D); propodeum mostly smooth with a medial longitudinal carina, meeting transverse carinae two thirds down propodeum in a 'Y' shaped configuration.

***Wings*:** pterostigma 2.5 (2.4) × longer than wide, with outer edge rounded and protruding slightly from wing outline (Fig. 4C); length of vein 2RS 4.4 (4.3) × r-rs, vein 2-M 1.3 (2.0) × longer than r-rs (Fig. 4C).

***Legs*:** hind coxa, femur and tarsus densely setose, except for basal hind coxa sparsely setose; length of hind femur 2.1 (1.7) × hind basitarsus; length of hind tibia 3.1 (2.6) × hind basitarsus.

***Metasoma*:** mesosoma 0.4 (0.5) × body length; T1 approximately 2.1 (2.4) × longer than maximum width, teardrop shaped, almost ovoid apically; T1 Smooth, with a few setae distally; ovipositor sheaths short, 3.8 (5.0) × longer than wide, densely setose (Fig. 4G).

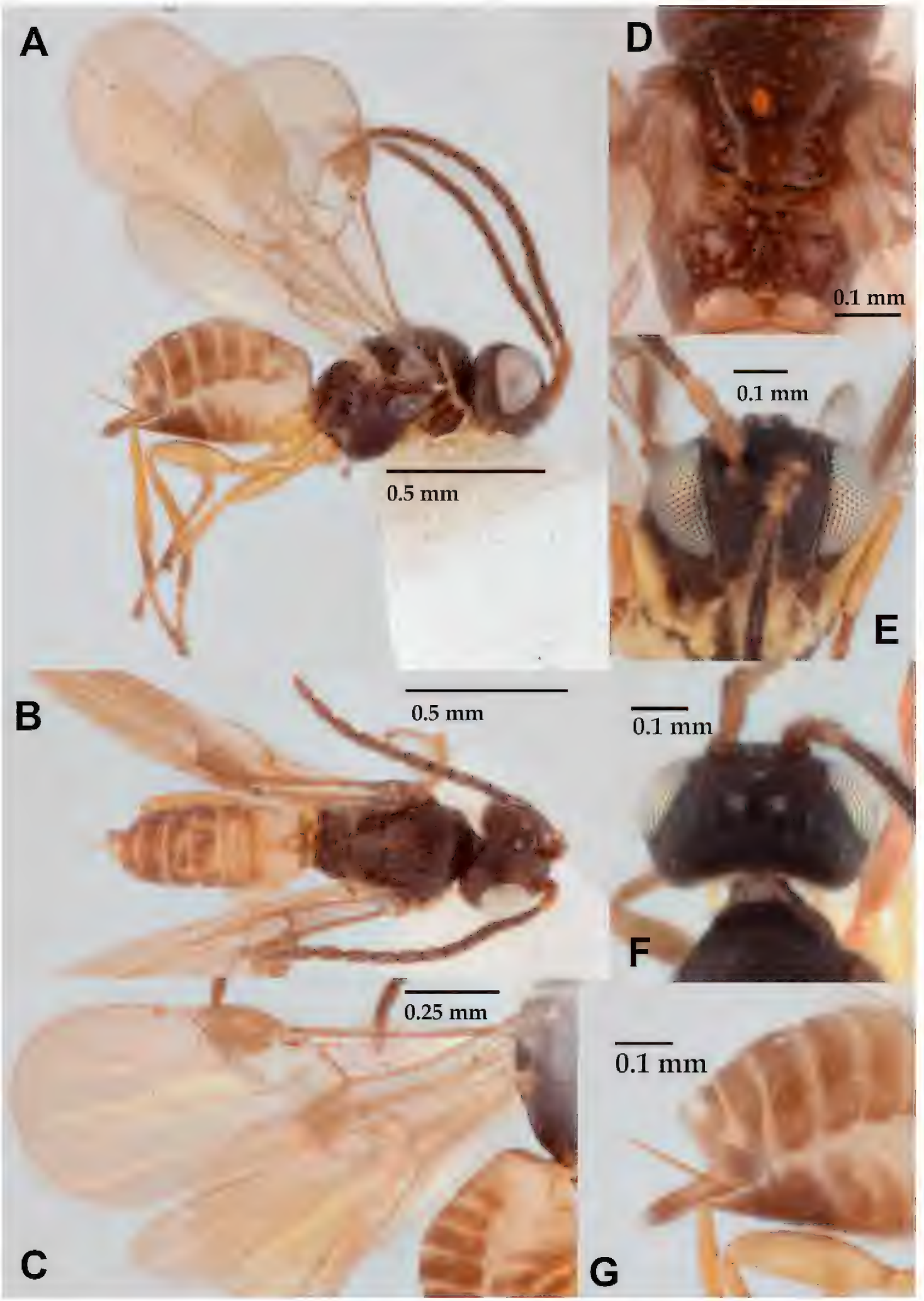

Figure 4. *Mirax supremus*: holotype (**A–D, G**), female paratype (**E, F**) **A** lateral habitus **B** dorsal habitus **C** wings **D** dorsal view of scutellar medio-posterior depressions and propodeum **E** anterior head **F** dorsal head **G** lateral posterior metasoma showing ovipositor and sheaths.

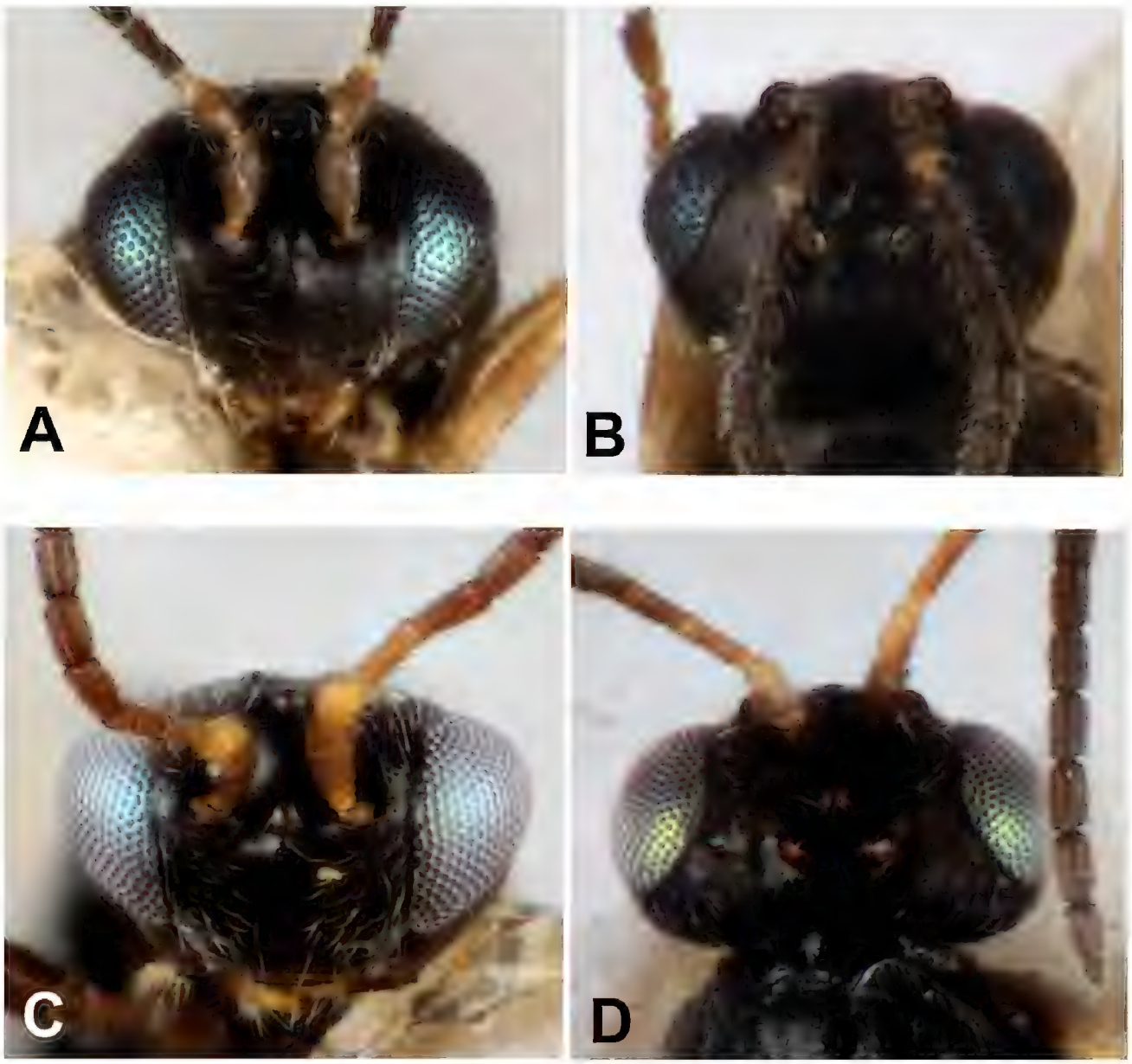

Figure 5. A, B *Mirax cowellensis* holotype **A** anterior head **B** dorsal head **C, D** *Mirax trianguliceps* holotype **C** anterior head **D** dorsal head.

Male. Appears similar to the female, except for the following: fore wing 1.7 mm; mesosoma dark, brown basally; T1 brown with dark margin; T2 dark; antennae longer than the body; penultimate flagellomere 2.9× longer than wide; POD:POL:OOL 1:1.3:2.7; head width 1.4× face height; dorsal medial head length 1.4× longer than dorsal eye length; pterostigma 3.2× longer than wide; length of vein 2RS 5.3× r-rs, vein 2-M 1.9× longer than r-rs; T1 2.8× longer than wide.

Remarks. The holotype and female paratype were collected in Queensland, and may be expected to show lighter overall colouration compared to specimens collected from other states in Australia (based on observations of related braconid subfamilies). At time of publication, this species forms BOLD BIN: BOLD:ADS1803 and is 4.33% divergent from its nearest neighbour on BOLD based on *COI*.

Etymology. The species epithet 'supremus' is a Latin masculine adjective meaning 'highest' or 'loftiest', to represent the term 'pinnacle'. This epithet was selected by the year 8–9 students of Beerwah State High School who collected the holotype, to celebrate the students being part of the Pinnacle Class program at their school. Female

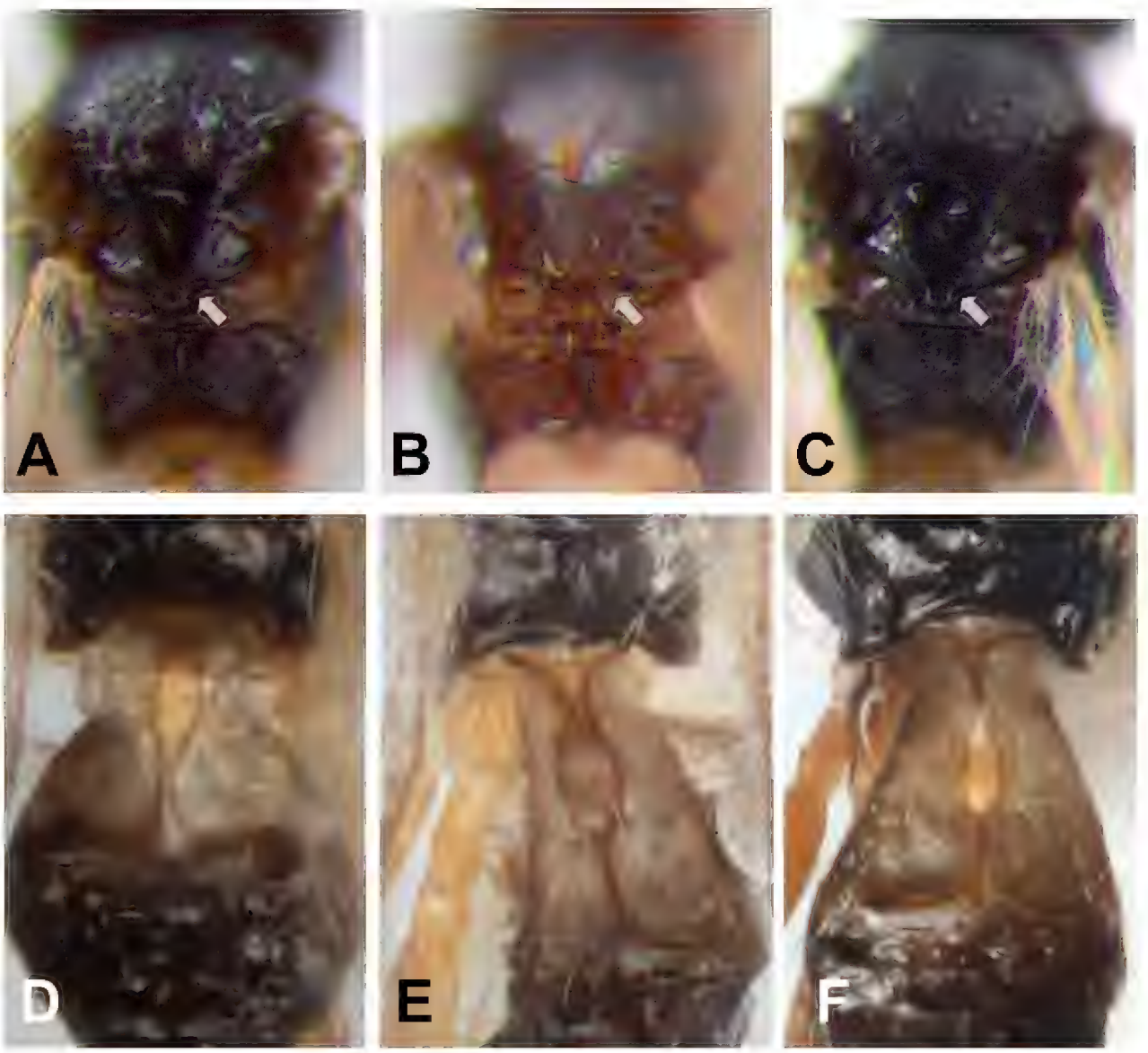

Figure 6. A, D *Mirax caelicus* holotype **B** *M. supremus* holotype **E** *M supremus* male paratype **C, F** *M. arcisensis* holotype **A–C** T1 **D–F** dorsal mesosoma, with grey arrow indicating the location of scutellar medio-posterior depression.

type specimens are deposited at the Queensland Museum, Australia, and the male paratype is deposited at the Australian National Insect Collection, Australia.

Distribution. This species is currently known from Queensland and New South Wales, Australia. It is likely to be distributed along the east coast of Australia, however may also be found in other regions. Further sampling is required to determine an accurate distribution for this species.

Discussion

Collection of Miracinae in Australia (at least in Malaise trap samples) appears to be quite rare, with only three miracine wasps collected and barcoded during the wide-reaching collection efforts of the *Insect Investigators* project, which resulted in collection of over 4800 Hymenoptera specimens, including 407 braconids (https://insectinvestigators.com.au/). Despite the rarity of collection, it appears likely that Australia is home to a diversity of Miracinae, and many more undescribed species remain uncollected, or unidentified in existing public collections. Considering their rarity in Malaise trap samples, rearing of miracine

wasps from their leaf-mining hosts may be an effective method for more rapid documentation of Australian Miracinae. Obtaining accurate host records would additionally provide improved knowledge of miracine wasp ecology and possible biological control applications.

The decision to treat *Centistidea* as a synonym of *Mirax* here was made as a conservative approach considering preliminary molecular results and observed variation and overlap in morphological characters used for diagnosis of genera of Miracinae. As observed in previous work (Slater-Baker et al. 2022), tree reconstruction analysis of *COI* barcodes appears to support the notion that current generic definitions within Miracinae require revision. Established morphological characters for diagnosing genera of Miracinae – mostly being the sculpture of the propodeum, impression of notauli, and size and separation of the scutellar medio-posterior depressions – appear to separate currently recognised genera in most cases (Liu and Polaszek 2024). However, Miracinae appears to display considerable variation in these characters (Ranjith et al. 2019; Ranjith et al. 2023; Liu and Polaszek 2024), which is likely to be expanded with further documentation of the subfamily, especially in poorly documented regions including Australia. Phylogenetic analysis of genomic data for a broad range of miracine wasps would be beneficial to better understand taxonomic relationships within the subfamily, and refine morphological diagnoses.

Whilst we recognise that in light of potential future sampling improvements, the taxonomic treatments used here are likely to require future revisions, we instead have chosen to focus on species-level documentation of Australian Miracinae. Our approach allows for the subfamily Miracinae to be recognised for its presence and diversity within Australia.

Outreach outcomes

Engagement with schools provided an opportunity to inspire young scientists, foster a sense of connection to local biodiversity, and raise awareness of contemporary taxonomic work. Students were interested in the unique life cycle of parasitoid wasps, and brainstormed names related to their biology (e.g erucaperditor – 'destroyer of caterpillars' or internifigens – 'to kill from inside'). Students were keen to draw on their community and local environment as inspiration for their name ideas. We noted that workshops with an in-person component seemed more engaging for students than online-only sessions, though online-only sessions still achieved their goal of connecting students with taxonomists and involving them throughout the taxonomic process. Working with schools generated opportunities for engagement with the broader community through media releases highlighting the kids' involvement in the project, and promoting a message of the importance of biodiversity discovery (Arriaga-Jiménez 2023; Australian Citizen Science Association 2023; Denmark Bullitin 2023; UniSC News 2023). Working directly with taxonomists fostered students' sense of their tangible contribution to the documentation of Australian biodiversity, while media coverage further reinforced the importance of their role in the project. Forming a strong and consistent line of communication with the schools involved, as well as media contacts, was essential to this process, which was reflected in the timeframes (11 to 27 weeks) required for the collaborative naming process.

Acknowledgements

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Supplementary material 1

Specimen collection and COI DNA barcode information

Authors: Mollie-Rosae Slater-Baker, Michelle Guzik, Juanita Rodriguez, Erinn Fagan-Jeffries

Data type: xlsx

Explanation note: Specimen collection and COI DNA barcode information for all available miracine wasps on the Barcode of Life Database as of May 2023 (n = 451), excluding sequences flagged as contaminants, containing stop codons, misidentifications/errors, and sequences of < 100 bp length. Data was downloaded directly from BOLD systems, with additional information added for Australian species (highlighted green).

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Link: https://doi.org/10.3897/jhr.98.137806.suppl1

Supplementary material 2

Distance matrix comparing % difference in *COI* DNA barcodes for three new species of Australian Miracinae, compared to other Australian Miracinae specimens

Authors: Mollie-Rosae Slater-Baker, Michelle Guzik, Juanita Rodriguez, Erinn Fagan-Jeffries

Data type: xlsx

Explanation note: Distances are displayed in each cell separated by ' / ' with simple distance followed by K2P corrected distances. Simple distances were calculated in Geneious Prime version 2024.0.5, from an alignment of Miracinae *COI* barcodes producing using MAFFT v7.490. K2P corrected distances were calculated in MEGA 11 version 11.0.13. Cells shaded green indicate *COI* difference values < 2% (species delimitation threshold), yellow indicates values between 2–10%, and red indicates values > 10%. Cells are shaded according to the highest value of the two distance methods used.

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Link: https://doi.org/10.3897/jhr.98.137806.suppl2

Supplementary material 3

Terminology URI table of Hymenoptera Anatomy Ontology preferred terms, used for the description of Australian Miracinae

Authors: Mollie-Rosae Slater-Baker, Michelle Guzik, Juanita Rodriguez, Erinn Fagan-Jeffries

Data type: xlsx

Explanation note: The table was produced using the analyse feature on the Hymenoptera Anatomy Ontology website.

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Link: https://doi.org/10.3897/jhr.98.137806.suppl3

Supplementary material 4

Maximum Likelihood *COI* tree of Miracinae produced using IQ-TREE v2.3.5

Authors: Mollie-Rosae Slater-Baker, Michelle Guzik, Juanita Rodriguez, Erinn Fagan-Jeffries

Data type: pdf

Explanation note: Maximum Likelihood *COI* tree of Miracinae produced using IQ-TREE v2.3.5, based on a MAFFT alignment of all miracine wasp *COI* DNA barcodes on BOLD as of May 2023 (n = 451), excluding sequences flagged as contaminants, containing stop codons, misidentifications/errors, and sequences of < 100 bp length. Node support is displayed as SH-aLRT values and Ultrafast bootstrap support values separated by '/' respectively. Sequences for specimens collected in Australia are highlighted green.

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Link: https://doi.org/10.3897/jhr.98.137806.suppl4

Supplementary material 5

Two alternative Maximum Likelihood analyses of COI of Miracinae produced using the IQ-TREE v2.3.5IQ-Tree web server

Authors: Mollie-Rosae Slater-Baker, Michelle Guzik, Juanita Rodriguez, Erinn Fagan-Jeffries

Data type: pdf

Explanation note: Trees are based on a MAFFT alignment of all miracine wasp COI DNA barcodes on BOLD as of May 2023 (n = 451), excluding sequences flagged as contaminants, containing stop codons, misidentifications/errors, and sequences of < 100 bp length. Node support is displayed as SH-aLRT values and Ultrafast bootstrap support values separated by '/' respectively. Sequences for specimens collected in Australia are highlighted green.

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Link: https://doi.org/10.3897/jhr.98.137806.suppl5

Supplementary material 6

Species delimitation results for Miracinae produced using the ASAP web server

Authors: Mollie-Rosae Slater-Baker, Michelle Guzik, Juanita Rodriguez, Erinn Fagan-Jeffries

Data type: pdf

Explanation note: The analysis was run using both K2P distances and simple distances (P-distances) to compare results: **A** results for simple distances **B** results for K2P distances. The partitions with the lowest ASAP score (selected as the best partition after consideration of probability values for alternative partitions) are highlighted. Sequences for Australian Miracinae are highlighted green.

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